EXHIBIT B

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AMPLIGASE™ Thermostable DNA Ligase Kit

AMPLIGASE™ Thermostable DNA Ligase is an NAD-requiring ligase derived from a thermophilic bacterium. The enzyme is active over a wide temperature range (15°-85°C), with the optimal temperature for ligation dependent upon the nature and temperature stability of the DNA structures to be ligated. **AMPLIGASE™ Ligase** is most suitable for use in **Nick Ligation Assays**, for synthesis of genes using overlapping oligodeoxynucleotides, or for other manipulations of DNA which involve ligation of nicks in duplex DNA. Detection of specific sequences in a sample DNA by Nick Ligation Assay is based on the ability of oligodeoxynucleotides to ligate when their ends are adjacent to each other following hybridization to a denatured DNA template. If the nucleotides at the ligation junction are not correctly base-paired with the template DNA, ligation will not occur. Under the appropriate conditions, even a single base mismatch with the template should be detected. Ligation assays with non-thermostable ligases have been reported (1-4). The thermostability of **AMPLIGASE™ Ligase** provides the advantages that: (a) the researcher can use the temperature which gives the optimal hybridization stringency for his reactions; and (b) the researcher can amplify the amount of ligation product formed by cycling the temperature between reaction conditions and DNA denaturation conditions, allowing successive cycles of ligation to occur. Backgrounds in Nick Ligation Assays are very low because **AMPLIGASE™ Ligase** has no detectable activity in ligating blunt-ended DNA fragments and is as much as 10,000-fold less active than T4 DNA Ligase in ligating four-base-pair cohesive ends generated by restriction enzymes when the ligations are carried out at or below the melting temperatures for such ends. The researcher should note that small amounts of dimers or multimers may be formed during some chemical syntheses of oligodeoxynucleotides. Since these contaminants can result in background signals in Nick Ligation Assays, the researcher should be sure that his oligo purification procedure completely removes such contaminants.

The **AMPLIGASE™ Kit** consists of 5000 units of **AMPLIGASE™ Thermostable DNA Ligase**, a 10X Reaction Buffer, and 5 ug of SmaI- and SalI-digested bacteriophage lambda DNA as a control. Control DNA allows the researcher to simultaneously observe ligation of the lambda cos site, which is analogous to a nick ligation, and to verify the absence of ligation of SmaI ends and minimal ligation of SalI cohesive ends.

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Unit Definition: One unit catalyzes the ligation of 50 % of the cos sites in one microgram of SmaI- and SalI-digested bacteriophage lambda DNA in one minute at 45°C.

Activity Assay: The Activity Assay is carried out with one microgram of SmaI- and SalI-digested bacteriophage lambda DNA in 50 ul of 1X Reaction Buffer, consisting of 20 mM Tris-HCl, pH 7.6, 25 mM potassium acetate, 10 mM magnesium acetate, 10 mM dithiothreitol, 0.6 mM NAD, and 0.1% Triton X-100. The reaction is followed by agarose gel electrophoresis.

Storage Solution: 50 % glycerol solution containing 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 0.1% Triton X-100, and 1 mM dithiothreitol. **Store at -20°C in a non-frost-free freezer.**

Stability: Enzyme activity was unchanged after incubation in 1X Reaction Buffer for one week at 37°C. The half-life of the enzyme in 1X Reaction Buffer is about 48 hours at 65°C and greater than 1 hour at 95°C.

Contaminating Activity Assays: No DNase activity is detected when 100 units of **AMPLIGASE™ Ligase** are incubated for 16 hours at 70°C under standard Activity Assay conditions with one microgram of supercoiled plasmid DNA, single-stranded M13mp18 DNA, or 5'-phosphorylated or unphosphorylated oligodeoxynucleotides, as judged by agarose or polyacrylamide gel analyses.

References:

1. Landegren, U., Kaiser, R., Sanders, J., and Hood, L. A ligase-mediated gene detection technique. Science, 241, 1077-1080, 1988.

2. Landegren, U., Kaiser, R., Caskey, C.T., and Hood, L. DNA diagnostics - Molecular techniques and automation. Science, 242, 229-237, 1988.

3. Wu, D.Y. and Wallace, R.B. Specificity of the nick-closing activity of bacteriophage T4 DNA ligase. Gene, 76, 245-254, 1989.

4. Wu, D.Y. and Wallace, R.B. The ligation amplification reaction (LAR) - Specific DNA sequences using sequential rounds of template-dependent ligation. Genomics, 4, 560-569, 1989.

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